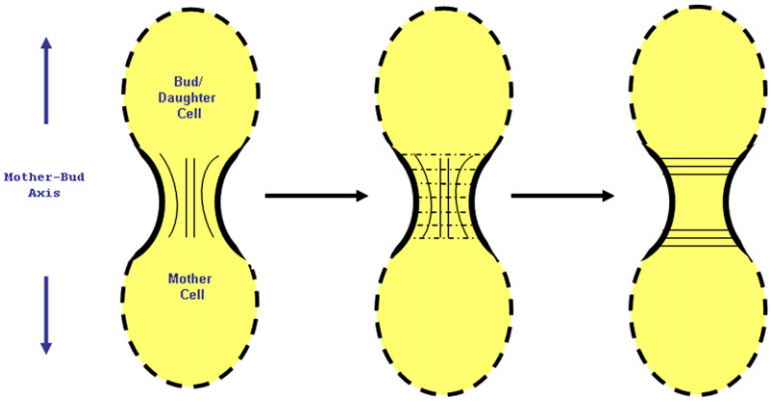

Figure 1. Current Model of Septin Filament Organization

Septin filaments initially associate in longitudinal orientation, parallel to the mother-bud axis in the narrow bridge separating mother and bud cells during bud growth (left). Coincident with septin phosphorylation, this structure becomes a static, rigid structure (middle). Following activation of Tem1 and dephosphorylation, the hourglass splits into two rings, concomitant with an overall rearrangement of septin filaments to a circumferential organization (right).

hourglass. Using in-vitro-purified septin filaments, they were able to define the dipole direction relative to the filament axis, and therefore concluded that the septin filaments reorient from being aligned in parallel to the mother-bud neck to being aligned perpendicular to it (see Figure 1).

It is remarkable that this 90° rotation of a previously constrained and static septin scaffold occurs precisely at the time of septin hourglass splitting and coincident with a return of the septins to a fluid-like state. The authors speculate that this spectacular rearrangement of septin filament orientation suggests a mechanical role for the septins in cytokinesis. In addition, it may explain many of the conflicting imaging results to date in the literature, as well as the distinct change in cellular machinery recruited to the septin scaffold at this time in cell division (Dobbelaere and Barral, 2004).

This elegant and insightful application of polarized microscopy exemplifies the potential of this technique as a tool for the study of septin filament regulation and function, particularly in simple, easily manipulated organisms such as yeast. A plethora of studies will now be possible involving the comparison of septin function prior to and after hourglass splitting in wild-type and mutant backgrounds. It would be of great interest to determine the relationship between filament orientation and their role as diffusion barriers. Additional studies will also be needed to understand the intriguing relationships between septin orientation, fluidity, and phosphorylation. A more challenging task will be to determine if similar rotational events occur in septin structures in less easily manipulated organisms such as mammals.

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Dissecting Mitochondrial Fusion

Because mitochondria have outer and inner membranes, the fusion of two mitochondria requires the coordinated fusion of four lipid bilayers. Fusion of the outer membranes requires mitofusins. A new study published in *Cell* (Meeusen et al., 2006) shows that inner membrane fusion requires the dynamin-related protein Mgm1/OPA1.

Mitochondrial dynamics has emerged in recent years as an important factor controlling the function of mitochondria (Chan, 2006). The fusion and fission of these

organelles regulate their number, size, and organization. In addition, it is now clear that beyond such control of mitochondrial morphology, mitochondrial dynamics has important roles in regulating their function. Loss of mitochondrial fusion results in autonomous organelles with heterogeneous membrane potential and poor ability to respire. Mutations in Mfn2 and OPA1, two molecules important for mitochondrial fusion, are associated with Charcot-Marie-Tooth type 2A and dominant optic atrophy, two neurodegenerative diseases. In addition, mitochondrial fission accompanies and facilitates many forms of apoptosis.

Yeast genetic studies on mitochondrial fusion have identified several key proteins, including the mitofusin Fzo1, the dynamin-related protein Mgm1, and the outer membrane protein Ugo1. To address the molecular action of these requirements, Nunnari and colleagues have developed an in vitro mitochondrial fusion assay (Meeusen et al., 2004). In the first step of this assay, mitochondria isolated from two yeast strains are mixed, centrifuged, and incubated in the absence of exogenous GTP. When mitochondria from wild-type cells are used, this first step results in the formation of intermediates that show outer membrane fusion. Progression of such intermediates to full fusion requires suspension of the mitochondria into a solution containing GTP and an energy regenerating system. Given the rapid rate at which mitochondria can fuse in vivo, it is clear that outer membrane and inner membrane fusion are highly coordinated. However, outer and inner membrane fusion in the in vitro assay have different requirements for GTP and energy, and react differently to pharmacological agents, implying that the two processes are mechanistically distinct. Experiments with cultured cells also support the view that outer membrane fusion and inner membrane fusion are distinct processes (Malka et al., 2005).

In the in vitro assay, Fzo1 is required for outer membrane fusion (Meeusen et al., 2004). Fzo1 is an excellent candidate for mediating outer membrane fusion, given that it is located in the mitochondrial outer membrane and is capable of forming homo-oligomeric complexes. Fzo1 is required in adjacent membranes for fusion in the in vitro assay. Moreover, the mammalian orthologs of Fzo1, the mitofusins Mfn1 and Mfn2, are also required in vivo on adjacent mitochondrial membranes during fusion. Biochemical and structural studies indicate the mitofusins function to tether mitochondria together at an early stage of fusion (Koshiba et al., 2004).

What is responsible for the fusion of the mitochondrial inner membrane? A new study published in *Cell* by Nunnari and colleagues provides compelling evidence that Mgm1 is required for inner membrane fusion (Meeusen et al., 2006). When Mgm1 function is blocked through the use of temperature-sensitive alleles, outer membrane fusion occurs in vitro but is not followed by inner membrane fusion. Importantly, this selective defect in inner membrane fusion also appears to happen in vivo. Given the association of Mgm1 with the mitochondrial inner membrane, it is certainly a good candidate for mediating inner membrane fusion. However, previous studies of an Mgm1 null allele documented a loss of both outer and inner membrane fusion (Sesaki et al., 2003). One concern is that the temperature-sensitive alleles of the present study may have selective or partial defects in mitochondrial fusion. However, it seems more likely that this discrepancy is due to secondary defects that accumulate in mitochondria constitutively lacking Mgm1. Indeed, Nunnari and colleagues find that fusion intermediates can be observed in yeast harboring temperature-sensitive alleles of Mgm1 only at short time intervals after shifting to the nonpermissive temperature. With prolonged loss of Mgm1, additional abnormalities in mitochondrial structure accumulate.

This important study raises a number of interesting issues. After outer membrane fusion, does Mgm1 act as a fusogenic molecule that brings opposing mitochondrial inner membranes together and mediates their fusion? The answer to this question awaits future studies, but some observations suggest the exciting possibility that Mgm1 might play a direct role in the fusion process. In fusion assays between mitochondria from wild-type cells and *mgm1* mutant cells, there is reduced fusion, suggesting that interactions in *trans* are important for high fusion activity (Meeusen et al., 2006). However, this *trans* requirement is not absolute, in contrast to mitofusins (Koshiba et al., 2004). If Mgm1 is indeed a component of the inner membrane fusion machinery, it will be important to understand how its activity is coordinated with Fzo1 activity to facilitate rapid fusion of both membranes. In yeast, a fraction of Fzo1 is linked to Mgm1 through the outer membrane protein Ugo1, but the ortholog of Ugo1 in mammals is unknown.

An alternative model is that Mgm1 is primarily required for proper cristae structure (Frezza et al., 2006; Griparic et al., 2004; Meeusen et al., 2006; Olichon et al., 2003). In addition to cristae defects, loss of OPA1 in mammalian cells results in loss of membrane potential and respiratory activity (Chen et al., 2005; Olichon et al., 2003), defects that would further interfere with mitochondrial inner membrane fusion. As a result, the defects in inner membrane fusion may be a secondary consequence of structural defects. It is also possible that Mgm1/OPA1 has multiple functions.

A key issue for future studies is how Mgm1/OPA1 acts at a molecular level. Mgm1/OPA1 is a dynamin-related GTPase, and classical dynamins have well-known functions in tubulation and constriction of lipid membranes. Although such membrane-deforming properties have yet to be documented for Mgm1, it is widely assumed to be critical for its function. A perplexing aspect is that other dynamin family members tubulate membranes by constricting from the outside surface of the tubule. In contrast, because Mgm1 is associated with the inner membrane, it is located on the inside of cristae tubules. Therefore, if Mgm1 is involved in tubulation of the cristae, it is operating in a manner topologically different from classical dynamins. This reverse topology is also present in a recent model that OPA1 might regulate cristae junctions (Frezza et al., 2006). An alternative possibility is that, during inner membrane fusion, Mgm1 mediates tubulation of the inner membranes outward from the mitochondria, allowing opposing mitochondria to reach each other. In such a model, the membrane topology of Mgm1/OPA1 would be the same as in classical dynamins. Resolution of this issue will likely require

development of an in vitro assay for OPA1 interactions with mitochondrial membranes.

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Myosin I and Actin Dynamics: The Frogs Weigh In

In this issue of *Developmental Cell*, Sokac et al. (2006) describe an intriguing new role for an actin-based motor protein in restraining actin polymerization during endocytosis in *Xenopus* oocytes.

The class I myosins (M1s) are a widespread and conserved group of monomeric actin-based motor proteins (Krendel and Mooseker, 2005). They have a strikingly diverse range of cellular roles, from ion channel gating to membrane trafficking. While it was initially envisioned that they might drive various intracellular organelles along actin tracks, this does not seem to be the case. Rather, several M1s have been found to play a role in controlling membrane-associated actin polymerization that contributes to vesicle scission and propulsion. A striking and unexpected example of a link between a M1 and actin polymerization has now emerged from studies in the *Xenopus* oocyte (Sokac et al., 2006).

Fertilization of a *Xenopus* oocyte is rapidly followed by the exocytosis of a large number of cortical granules (CGs) docked near the membrane surface. The expelled contents of these granules are used to form the fertilization envelope that serves as a block to polyspermy. This massive exocytic event also causes an increase in total surface membrane that is then reduced via compensatory endocytosis (for review see Sokac and Bement, 2006). This endocytic event relies on the recruitment of an actin coat around the vesicle membrane and the subsequent constriction of this coat results in scission of the nascent endocytic vesicle from the membrane and vesicle compression. The expression of a gene encoding one particular *Xenopus* myosin, Myo1c, is selectively upregulated during oogenesis and meiotic maturation (an event that readies the egg for fertilization), suggesting a specific role for Myo1c in meiotic maturation or early development (Sokac et al., 2006). GFP-Myo1c is colocalized with actin to the cortical membrane in oocytes, but following activation it is strikingly redistributed to exocytic CG membranes. Interestingly, this recruitment precedes that of actin, suggesting that Myo1c could serve to nucleate or stimulate actin polymerization at the membrane of exocytosing CGs.

The M1 tail region possesses sites for lipids and interaction with binding partners that most likely direct subcellular localization. Overexpression of the Myo1c tail region plus the neck region in oocytes results in the displacement of the full-length myosin from the plasma membrane and causes a block in CG exocytosis (Sokac et al., 2006). This inhibition of exocytosis corresponds with slowed closure of the fusion pore that occurs during compensatory endocytosis, an actin-dependent process. Inspection of the stalled large endosomes (LEs) revealed the presence of an actin coat surrounding each, as expected, but a failure of the coats to compress. Gaps are observed between actin and the endosome membrane and "fingers" of actin extend from the plasma membrane into the cytosol. Surprisingly, the excess Myo1c tail also promotes the formation of overly long cortical microvilli and ectopic actin comet tails in the cortical region. These changes in actin behavior are also observed in oocytes with reduced Myo1c expression caused by the injection of antisense morpholinos. They are also fully rescued by coexpression of full-length Myo1c as well as depolymerization of actin, as might be expected if inappropriate actin polymerization in the cortex presents a barrier to CG or LE movement. The unexpected conclusion from the accumulated data is that Myo1c is not required to promote actin polymerization but rather to restrict or inhibit it.

The link between M1 and actin dynamics in the *Xenopus* oocyte is a new twist on a previously described relationship. The amoeboid-type M1s in lower eukaryotes, e.g., *Dictyostelium* myoB and yeast Myo3p or Myo5p,